IMPACT OF PREHARVEST AND POSTHARVEST TREATMENT COMBINATIONS ON INCREASE OF STILBENE CONTENT IN GRAPE

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Abstract

Aims : Stilbene-enriched grape is an interesting new food product with numerous health-promoting properties, mainly due to its high added-value compound content, notably resveratrol. The aim of this study was to evaluate the effects of different elicitors, alone or in combination with ultraviolet C light (UVC) postharvest treatment, on stilbene concentration in grapes.

Methods and results : Three preharvest treatments were tested, namely benzothiadiazole, (BTH), methyl jasmonate (MEJA) and chitosan (CHIT). After harvesting, these treatments were combined with UVC postharvest treatment. The stilbene extraction method was validated method. Moreover, and grape quality was evaluated. Of the preharvest treatments, only BTH significantly increased *trans*-resveratrol concentration in grape, but this appears to be linked to a ripening delay. When pre- and postharvest treatments were combined, only the MEJA-UVC combination was successful in reducing by three days the day of maximum induction of stilbenes (*trans*-resveratrol and piceatannol).

Conclusion : The MEJA-UVC combination reached similar grape *trans*-resveratrol contents than UVC alone, but additionally the time to reach maximum *trans*-resveratrol after the UVC treatment was reduced by three days and therefore grape quality was preserved.

Significance and impact of the study : The achieved results provide a potential treatment combination that allows functional grapes to be obtained in a shorter period than with UVC light alone, making it more applicable.

Key words : benzothiadiazole, chitosan, methyl jasmonate, resveratrol, piceatannol, viniferins, functional grape, ultraviolet C light

Résumé

Buts : Des baies de raisin enrichies en stilbènes constituent un produit alimentaire innovant à forte valeur ajoutée liée aux nombreux effets potentiellement bénéfiques sur la santé de ces composés et principalement du resvératrol. Le but de cette étude a été d'évaluer les effets de différents éliciteurs sur la concentration en stilbènes de la vigne. Ces éliciteurs ont été testés seuls ou combinés avec un traitement aux rayons ultraviolets C light (UVC) après la récolte.

Méthodes et résultats : Trois traitements pré-récolte ont été testés : benzothiodiazole, (BTH), méthyl jasmonate (MEJA) et chitosane (CHIT). Après récolte, ces traitements ont été combinés à une exposition aux UVC. La méthodologie d'extraction des stilbènes a été validée. De plus, la Après validation de la méthode a d'abord été validée, la qualité des baies a été évaluée. Parmi les traitements pré-récolte, seul celui au BTH augmente significativement la concentration en *trans*-resvératrol dans les baies avant et au moment de la récolte. Cependant, il apparaît que cela cet effet soit lié à un retard de maturité des baies. Lorsque des traitements avant et après récolte sont effectuéscombinés, seul le traitement MEJA-UVC a permis de réduire la durée conduisant à une concentration maximale en stilbènes (*trans*-resvératrol et piceatannol) de 3 jours.

Conclusion : La combinaison MEJA-UVC donne des concentrations en *trans*-resvératrol du même ordre de grandeur que le traitement par UVC seul. Cependant cette combinaison permet de réduire de trois jours le temps nécessaire pour avoir une concentration maximale en elleLa combinaison MEJA-UVC permet d'induire plus rapidement la production de *trans*-resveratrol et donc sans altérer les propriétés œnologiquesla qualité des baies de raisin sont conservée.

Signification et impact de l'étude : Les résultats obtenus conduisent à une combinaison de traitements qui permettent d'obtenir des baies enrichies plus rapidement que par le seul traitement par UVC. la production de baies de raisin enrichies en stilbènes.

Mots clés : benzothiadiazole, chitosane, méthyl jasmonate, resvératrol, piceatannol, viniférines, raisin fonctionnel, rayons ultraviolets C light

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INTRODUCTION

The role of grape and wine in the human diet has been widely studied in recent years. Beneficial health properties such as antioxidant, anticancer, cardioprotective, anti-inflammatory, antibacterial and antihistaminic activities, among others, are attributed to them. These properties seem to be mainly related to polyphenols, a group in which stilbenes (in particular *trans*-resveratrol) can be highlighted. The biological properties of resveratrol include cardioprotective, neuroprotective and anticancer actions (Guerrero *et al.*, 2009). In fact, *trans*-resveratrol seems to be one of the most promising compounds due to its bioactivity. Piceatannol and viniferins are stilbenes usually found in grape and wine at lower concentrations than resveratrol. Consequently, their bioactivity has received less attention, although some of their health-promoting properties have been investigated (Guerrero *et al.*, 2009).

Stilbene sources in diet are scarce and resveratrol is found in small quantities in peanuts, berries and grapes (skin and seeds) and their derivatives (juice and wine) only. Both grape and wine are considered to be the most important dietary sources of stilbenes. Resveratrol concentration in grape and wine oscillates from traces to 8.97 mg/Kg and traces to 14.3 mg/L, respectively (Guerrero *et al.*, 2009). Resveratrol concentration in grape and wine depends on many variables: grape variety, growing conditions, climate, harvest year and winemaking techniques. Stilbene concentration can be increased in plants because they are phytoalexins and, therefore, can be induced by different stresses, many of which have been studied. For example, elicitors such as benzothiadiazole (BTH), chitosan (CHIT), "*Botrytis cinerea*", methyl jasmonate (MEJA), jasmonic acid, salicylic acid, *β*-aminobutyric acid, ozone, aluminum chloride and ultraviolet C (UVC) light have been used to enhance nutraceutical grape properties (Fernández-Marín *et al.*, 2012; Cisneros-Zevallos, 2003). Among them, the following elicitors can be highlighted: BTH, MEJA and CHIT. BTH (figure 1A) is a functional analogue of the hormone-like compound salicylic acid; MEJA (figure 1B) is the most active derivative of jasmonic acid; and CHIT (figure 1C) is a natural polysaccharide with a polycationic nature that has numerous applications in agriculture. All of these have been used to increase stilbene content in grape berries (Larronde *et al.*, 2003; Iriti *et al.*, 2004; Bautista-Baños *et al.*, 2006), leaves (Larronde *et al.*, 2003) and cell cultures (Belhadj *et al.*, 2008). In terms of toxicity, at the doses and with the application method used in our study, BTH (Iriti *et al.*, 2004) and CHIT (Meng *et al.*, 2008) are completely degraded in plant tissues, with no persistence of residues. As for MEJA, it is a non-toxic compound found naturally in grape berries (Kondo & Fukuda, 2001).

UVC postharvest technology has been described as one of the most effective treatments to increase stilbene content in grape (Jeandet *et al.*, 1995; Cantos *et al.*, 2001). In fact, this treatment is currently being used for nutraceutical production (Patent WO/2002/085137; ES 2177465; http://www.acta-farma.com/productos/revidox/www.revidox.es). However, the application of UVC for the production of stilbene-enriched wines is conditioned by the number of days of storage between the time of UVC treatment and the time of winemaking (Guerrero *et al.*, 2010b).

All the above stresses or their combinations can be used to target the increase of health-promoting stilbenes. Synergistic effect on phytoalexin production has been described between MEJA and ethephon (Faurie *et al.*, 2009), CHIT and UVC (Romanazzi *et al.*, 2006), MEJA and UVC (Larronde *et al.*, 2003), MEJA and cyclodextrins (Lijavetzky *et al.*, 2008), and others (Cisneros-Zevallos, 2003).

The aim of this study was i) to investigate the efficiency of BTH, MEJA and CHIT preharvest treatments on both stilbene content and quality properties of grapes and ii) to find possible synergies between preharvest treatments and UVC postharvest treatment.

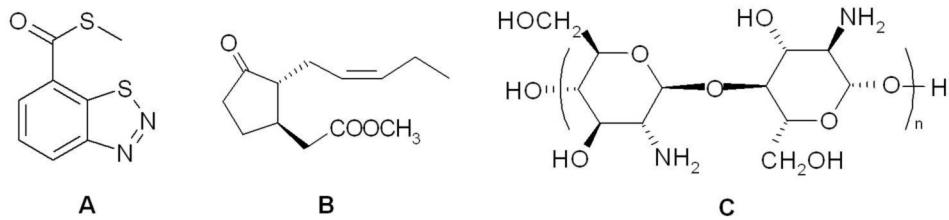

Figure 1 - Chemical structure of the elicitors: benzothiadiazole (BTH, A), chitosan (CHIT, B) and methyl jasmonate (MEJA, C).

MATERIALS AND METHODS

1. Reagents

trans-Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), piceatannol (3,3′,4,5′-tetrahydroxystilbene) and MEJA (methyl (1R,2R)-3-Oxo-2-(2Z)-2-pentenyl-cyclopentaneacetate) were purchased from Sigma-Aldrich (Madrid, Spain). BTH (benzo-(1,2,3)-thiadiazole-7-carbothionic acid S-methyl ester, BION 50 WG) was supplied by Syngenta (Madrid, Spain) and CHIT (poly β-(1→4) *N*-acetyl-D-glucosamine, Biorend R) by Idebio S.L. (Salamanca, Spain). Analytical grades of acetic acid, methanol, acetone, sodium hydrogen carbonate, ethyl acetate solvent and the emulsifier Tween 80 were supplied by Panreac (Barcelona, Spain). Ultrapure water from a Milli-Q system (Millipore, Bedford, MA) was used in this research.

2. Grapevine

Treatments were carried out in an experimental vineyard in "Jerez de la Frontera" (Cadiz, Spain), in 2010. The study was performed on four-year-old *Vitis vinifera* L. Syrah red wine grapevines grafted on 161-49C rootstock. A bilateral cordon training system trellised to a three-wire vertical system was used. Vine rows ran N-NW to S-SE and planting density was 2.40 m between rows and 1.20 m between vines. Six two-bud spurs (12 nodes) per vine were retained at pruning.

3. Open field preharvest treatments

All treatments were applied in triplicate using a completely randomized block design (10 vines per replicate). Plants were sprayed (50 mL per vine) early in the morning, at different times and concentrations (table 1).

The BTH treatment was performed in water suspension at 0.3 mM. Vines were sprayed three times (Iriti *et al.*, 2004): 20, 16 and 13 days before harvesting; control plants were treated the same way but sprayed with water only. Samples were taken every two days from the last treatment to harvest date (13 days). The MEJA treatment was performed in ethanol at 10 mM. Vines were sprayed three times (Vezzulli *et al.*, 2007): 20, 16 and 13 days before harvesting; control plants were treated the same way but sprayed with ethanol (98 %) only. Samples were taken every two days from the last treatment to harvest date (13 days).

A formulation based on a 1.25 % (w/v) solution of CHIT at 10 g/L was sprayed 10 days before harvesting; control plants were treated the same way with deionised water at pH 5.6 (Romanazzi *et al.*, 2006). Samples were taken every two days from treatment to harvest date (10 days).

After sampling, a batch of grapes from each treatment and control were peeled and kept at -80 °C until extraction.

4. UVC postharvest treatment

The preharvest treatments described above were combined with a UVC postharvest treatment. Harvested grapes were divided into two batches: the first batch (CT) came from control plants and was not treated; the second batch (UVC) came from preharvest treated grapes and was irradiated with UVC after harvest (figure 2), as previously described (Guerrero *et al.*, 2010a).

After UVC treatment, grapes were stored at 20 °C for one week to determine the "maximum day" (Dm) (Guerrero *et al.*, 2010a). The Dm parameter was defined as the number of days needed after UVC treatment to reach maximum *trans*-resveratrol concentration in grape. Grapes were sampled every day, peeled and kept at -80ºC until stilbene extraction (Guerrero *et al.*, 2010a).

Table 1. Preharvest treatment applications.

Treatment	Code	Solution	Concentration	Application	Sampling	Control
Benzothiadiazole	BTH	BTH in water solution	0.3 mM	20, 16 and 13 days before harvesting	Every other day from the last treatment to harvest (13 days)	Water
Methyl jasmonate	MEJA	MEJA in Ethanol	10 mM	20, 16 and 13 days before harvesting	Every other day from the last treatment to harvest (13 days)	Ethanol
Chitosan	CHIT	CHIT in water solution	10 g/L	10 days before harvesting	Every other day from treatment to harvest (10 days)	Deionised water at pH 5.6

5. Oenological parameters of grapes

Grape weight, Brix degree, total acidity, pH, tartaric acid, potassium, and maturity index (MI) were determined in grapes at harvest with official methods (OIV, 1990). Moreover, total anthocyanins, extractable anthocyanins, extractability, tannins, and total polyphenol index (TPI) were measured according to previously described methods (Saint-Cricq de Gaulejac *et al.*, 1998; Ribéreau-Gayon *et al.*, 2002).

6. Stilbene extraction

Stilbenes were extracted according to the method described previously by Bavaresco *et al.* (2001), with some modifications. Skin grapes were freeze-dried (Cryodos-80, TELSTAR, Spain) and 0.25 g of freeze-dried skin was extracted twice with 5 mL of sodium hydrogen carbonate (5 %) and 5 mL of ethyl acetate. The samples were ground using an Ultraturrax T-25 homogenizer (Janke & Kunkel, Ika-Labortechnik, Germany) and stirred at 1200 rpm for 20 minutes. The organic phase was first dried in a vacuum concentrator, then re-dissolved in 2 mL of methanol, and finally filtered through a 0.22-µm PVDF filter (Teknokroma, Barcelona, Spain). Extractions were performed in triplicate in darkness and at low temperature (wrapped in aluminum foil and immersed in an ice bath) to avoid oxidation and isomerisation reactions. Data are expressed in mg/Kg fresh weight (f.w.).

7. Identification and quantification of stilbenes

Stilbenes were quantified as described by Guerrero *et al.* (2010a). Samples (20 µL) were analysed by a Waters HPLC system with a model 1525 pump and a Waters 996 Photodiode Array Detector. Separations were performed on a Mediterranea sea18 column (Teknokroma, Barcelona, Spain) (RP-18, 25×0.46 cm; 5-µm particle size) and a guard column of the same material, at 30 °C. The mobile phases consisted of a water: methanol: acetic acid mixture, solvent A (88: 10:2) and solvent B (8: 90: 2). The elution programme involved gradient elution from 35 % B for 3 min to 50 % B after 10 min, 70 % B after 20 min and 100 % B after 23 to 28 min at a flow rate of 1 mL/min. *trans*-Resveratrol was quantified at 306 nm along with other stilbenes (piceatannol, viniferins (ε- and δ-viniferin) and total stilbenes (sum of the aforementioned), which were expressed as *trans*-resveratrol equivalent. These stilbenes had previously been identified by UPLC-MS-MS in our lab (Guerrero *et al.*, 2010a).

8. Method validation

A range of *trans*-resveratrol standards between 0.05 and 31.5 mg/L were prepared to evaluate linearity throughout calibration curve. The values of limit of detection (LOD) and limit of quantification (LOQ) were obtained as 3 and 10 times the signal-to-noise ratio/relationship. Intra-day and inter-day precision and accuracy were calculated. Accuracy was evaluated by means of recovery assays, as the relative error ((concentration found − concentration spiked)/(concentration spiked) × 100 %). *trans*-Resveratrol peak resolution in grape was carried out by adding standard solutions to the grape sample. Resolution was calculated as $Rs = 2\,\Delta t / (W1 + W2)$, where Δt is the difference in retention times between the two peaks; and W1 and W2 are the widths of the two peaks (in time units) (Schoenmakers, 1988).

The standard addition method (MOSA) (Harris, 1995) was applied to grape samples with 6 and 12 mg/L *trans*-resveratrol concentrations. Recovery = (b_{MOSA}/ b_{EC}) x 100, where b is the slope of calibration curve obtained with the spiked wine; MOSA is the Method of Standard Addition; and EC is the External

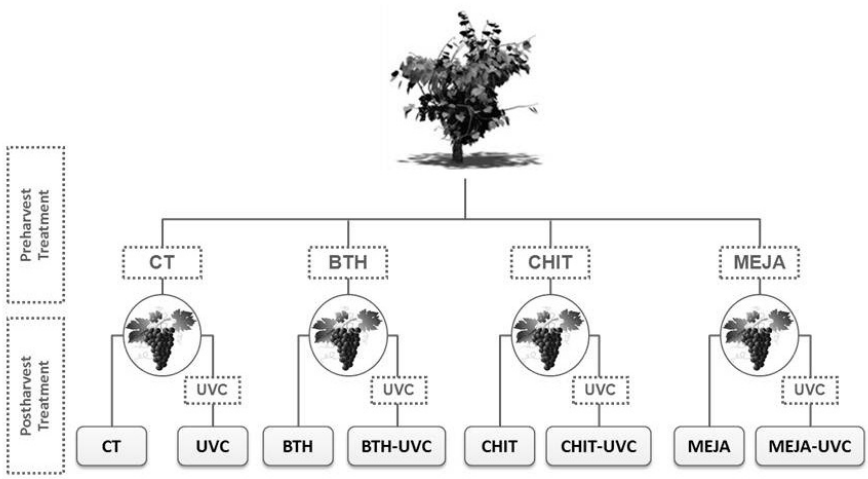

Figure 2. Diagram of grape treatments.

Calibration. Five replicates were performed in one working session in order to calculate intra-day repeatability. Analyses were performed on five different working sessions over a week to calculate intermediate inter-day accuracy. Both values are expressed as relative standard deviation (%RSD).

9. Statistical analysis

The analysis of the variance (ANOVA) and Least Significant Difference test (Tukey) were used with a significance level of $\alpha = 0.05$. Statistix version 8.0 (Analytical Software, Tallahassee, FL, USA) was used.

RESULTS AND DISCUSSION

1. Method validation

The method was validated in order to determine *trans*-resveratrol concentration (table 2). Linearity was assessed with standard solutions at concentrations ranging from 0.05 to 31.5 mg/L of *trans*-resveratrol standard, and performed in triplicate. Calibration curves were obtained by plotting the peak areas against different *trans*-resveratrol concentrations. LOD and LOQ were 0.033 mg/L and 0.102 mg/L, respectively. These data were consistent with those obtained by other authors (Goldberg *et al.*, 1996; Rodríguez-Bernaldo de Quirós *et al.*, 2009).

The recovery of *trans*-resveratrol was 103.74 % and 95.48 % and accuracy showed 93.53 % and 104.93 % for the two different standard concentrations, respectively. Both results are acceptable according to the AOAC (Huber, 1998). Intra-day variation was 4.87 % and inter-day 1.15 %. The *trans*-resveratrol peak resolution in wines was 0.02 and 0.39 time units for the whole set of samples.

Therefore, the method used was validated as reliable for the quantitative analysis of *trans*-resveratrol in grape samples, following AOAC recommendations (Huber, 1998).

2. Effect of preharvest treatments on oenological parameters of grapes

The effects of preharvest treatments on grape quality were studied at harvest. The oenological quality

Table 2 - Calibration curve, limit of detection, limit of quantification, recovery, accuracy and intra and interday variation of *trans*-resveratrol.

Intercept	Slope	R^2	Interval (mg/L)	LOD (mg/L)	LOQ (mg/L)	Recovery (%)		Accuracy (%)		Intraday variation RSD(%)	Interday variation RSD(%)
						Standard 6 mg/L	Standard 12 mg/L	Standard 6 mg/L	Standard 12 mg/L		
-36489	148359	0.997	0.05-31.5	0.033	0.102	103.74	95.48	93.53	104.93	4.87	1.15

LOD, Limit of Detection; LOQ, Limit of Quantitation

Table 3 - Oenological parameters of grapes at harvest.

	BTH		MEJA		CHIT	
	CT	TR	CT	TR	CT	TR
Grape weight (g)	1,79	1,79	1,88	1,92	2,03	1,94
Brix degree	18.5[a]	16.4[b]	17,2	17,8	16,8	16,8
Total Acidity (g/L TH_2)	6.44[b]	6.80[a]	6,63	6,93	7,42	7,07
pH	3.37[a]	3.26[b]	3,32	3,26	3,28	3,26
Tartaric acid (g/L)	7,18	7,36	6,61	7,26	6,97	6.22
Potassium (mg/L)	1622[a]	1550[b]	1641	1619	1560	1668
Total anthocyanins (mg/L)	296	281	263	347	254	225
Extractable anthocyanins (mg/L)	106	86	106	117	82	86
Extractability (%)	63	68	59	65	67	61
Tannins (g/L)	1,14	1,04	0,93	1,14	0,61	0,77
TPI	13,43	12,18	11,07	13,44	7,55	9,41
MI	27.64[a]	22.5[b]	24,59	24,53	21,43	22,49

CT, control; TR, treatment; TPI, total polyphenol Index; MI, maturation index.
Different letters mean significant differences between treatment (significant level p<0,05)

parameters were measured on a representative sample of each treatment and compared with control (table 3).

BTH treatment affected some of the oenological parameters of grape. Treated grape showed lower Brix degree, pH and potassium content and higher total acidity when compared with control ; therefore, it seemed that BTH treatment delayed the ripening process (MI, table 3). These data contrast with the findings of Ruiz-García *et al.* (2012), who described slight differences when performing the same treatment on Monastrell grapes. However, our results did not show any significant change in grape anthocyanin content (total and extractable). By contrast, Iriti *et al.* (2004) described a 2-fold increase of anthocyanin concentration (measured by HPLC) in Merlot treated with the same BTH concentration.

MEJA treatment did not modify any of the studied oenological parameters of grape (table 3), in agreement with other authors (Ruiz-García *et al.*, 2012). However, these authors have observed an increase in anthocyanin and flavonol content (measured by HPLC) in MEJA-treated grapes. Additionally, an increase in anthocyanin has been described when testing MEJA in grapevine cell cultures, especially when combined with sucrose (Belhadj *et al.*, 2008). Larronde *et al.* (2003) studied the effect of MEJA vapour preharvest treatment on grape stilbenes, but the oenological parameters were not measured.

CHIT treatment did not affect grape oenological parameters (table 3). Postharvest treatment with CHIT has been proposed as an edible coating in fruit because it decreases decay incidence and reduces respiration metabolism (Olivas & Barbosa-Cánovas, 2005). However, when CHIT treatment was studied in preharvest condition, it had no effect on oenological parameters of table grape (Meng *et al.*, 2008 ; Duxbury *et al.*, 2004), which is in agreement with our data.

3. Effect of preharvest treatments on *trans*-resveratrol content in grapes

Significant differences in *trans*-resveratrol content were observed in grapes treated with preharvest treatments. Since piceatannol and viniferins were below the LOQ, only *trans*-resveratrol was quantified (table 4). It has been described that no other stilbenes

Table 4. *trans*-Resveratrol concentration after preharvest treatments (mg/Kg f.w.)

	BTH		
	CT	TR	Induction (-fold)
4 days after TR	0.185	0.146	0.79
6 days after TR	0.060[b]	0.125[a]	2.08
8 days after TR	0.044[b]	0.109[a]	2.16
11 days after TR	0.030[b]	0.125[a]	4.17
12 days after TR (harvest)	0.093[b]	0.260[a]	2.79
	MEJA		
	CT	TR	Induction (-fold)
4 days after TR	0.123[b]	0.203[a]	1.65
6 days after TR	0.133	0.191	1.44
8 days after TR	0.106	0.143	1.35
11 days after TR	0.119[a]	0.065[b]	0.56
12 days after TR (harvest)	0.189[a]	0.079[b]	0.48
	CHIT		
	CT	TR	Induction (-fold)
2 days after TR	0.185	0.139	0.75
4 days after TR	0.106	0.174	1.64
7 days after TR	0.157	0.141	0.90
8 days after TR (harvest)	0.197	0.200	1.01

CT, control; TR, treatment; f.w., fresh weight
Different letters mean significant differences between treatment (significant level $p<0.05$)

were detected in grape samples when *trans*-resveratrol concentration was low (Guerrero *et al.*, 2010a).

BTH significantly increased *trans*-resveratrol content in grapes from the second sampling day (i.e., six days after treatment application) (table 4). It is worth mentioning that at harvest the *trans*-resveratrol content in treated grapes was 2.79 times higher with respect to control.

Maximum differences between MEJA-treated grapes and control were found after four days of treatment, when treated grapes contained 1.65-fold more resveratrol than control ones (table 4). However, *trans*-resveratrol subsequently decreased throughout ripening until harvest, with the decrease being more pronounced in MEJA-treated grapes than in control ones. Similar results were found in literature. In grapes treated with MEJA vapours, Larronde *et al.* (2003) described an increase in *trans*-resveratrol concentration during the 15 days after veraison but a marked decline during grape ripening. These data contrast with those obtained by other authors (Vezzulli *et al.*, 2007), in which MEJA treatment improved both resveratrol and ε-viniferin content of Barbera berries in an accumulative manner. In grapevine cell cultures, when MEJA (18 g/L) was added in the presence of sugars, both resveratrol and piceid contents significantly increased (Belhadj *et al.*, 2008). Other authors have described that when gaseous MEJA was used at very low concentration (0.09 mg/L) on Cabernet sauvignon grapes, resveratrol markedly increased (9-fold) before harvesting. Moreover, MEJA has been proposed as a very useful resveratrol-inductor in cell cultures when combined with cyclodextrin (Lijavetzky *et al.*, 2008).

CHIT treatment did not induce an increase in *trans*-resveratrol concentration with respect to control (table 4). When CHIT has been tested at low concentration over the canopy of Cabernet-Sauvignon vines, their phenolic content did not change (Duxbury *et al.*, 2004). In agreement with our data, the application of the same doses of CHIT on table grapes did not increase resveratrol content, but it has helped to control *Botrytis cinerea* infection (Romanazzi *et al.*, 2006). In cell culture (*V. vinifera* L. cv. Barbera), a concentration of CHIT five times higher than the one used in the current experiment led to an almost two times increase in *trans*-resveratrol content (Ferri *et al.*, 2009). Furthermore, CHIT was recently tested at the same concentration on another cell culture (*V. vinifera* L. cv. Italia). In this case, it was described as less effective for stilbene induction (Santamaria *et al.*, 2011), the origin of the cell culture type being the difference between these two studies. Ferri *et al.* (2009) obtained callus tissues from leaf petioles of *V. vinifera* L. cv. Barbera, whereas Santamaria *et al.* (2011) used stem and tendril explants from *V. vinifera* L. cv. Italia to induce callus formation.

Under these experimental conditions, it can be concluded that MEJA and CHIT preharvest treatments do not increase *trans*-resveratrol content in grape at harvesting, although using MEJA just before harvesting could be taken in consideration. BTH preharvest treatment increased *trans*-resveratrol content in grapes during ripening and also at harvesting, but this seems to be connected to ripening delay.

4. Combination of preharvest treatments with UVC postharvest treatment

Two requirements have been identified for achieving stilbene-enriched-wines using UVC: first, wine grape should show high induction capacity and second, Dm (minimum number of days to reach maximum stilbene concentration) should be as short as possible in order to preserve grape quality (Guerrero *et al.*, 2010b). This is the weak point of the UVC treatment since Dm is around 5-7 days depending on variety (Guerrero *et al.*, 2010a). In the present study, Syrah was chosen for its high resveratrol induction capacity, however, its Dm is 7 days (Guerrero *et al.*, 2010a). This period is too long for conserving grape quality until winemaking.

In this sense, we hypothesise that treatment combination (preharvest and UVC postharvest) could increase grape stilbene content due to synergistic effect. Moreover, treatment combination might reduce the induction period, and therefore the Dm period, by previous activation of the stilbene production metabolism through preharvest elicitors.

Synergistic effects have been described between CHIT and UVC irradiation in table grapes

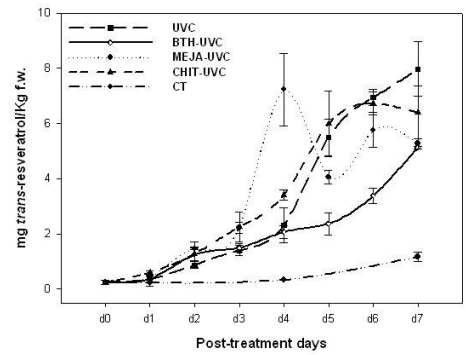

Figure 3. *trans*-Resveratrol concentration in grapes during the 7 days after treatment combinations.

(Romanazzi *et al.*, 2006) and MEJA and UVC in cell cultures (Larronde *et al.*, 2003 ; Zhang *et al.*, 2002). In the present study, the combination of preharvest treatments in vineyard (BTH, MEJA and CHIT) with UVC postharvest treatment was tested in grapes. Once ripeness was reached, grapes from the three preharvest treatments were harvested and immediately treated with UVC. Controls did not receive UVC treatment (figure 2).

The evolution of *trans*-resveratrol, piceatannol, ε- and δ-viniferins, and stilbenes after UVC treatment was followed daily for one week to determine Dm (figures 3 and 4).

When monitoring stilbene induction in grapes for seven days after the different treatment combinations, an increase in *trans*-resveratrol, piceatannol, and ε- and δ-viniferin content (hereafter viniferins) was observed in all treated grapes. These stilbenes have previously been identified by UPLC-MS-MS in our lab (Guerrero *et al.*, 2010a). Moreover, it was found that there were two different days on which *trans*-resveratrol reached maximum concentration, depending on treatment (fourth and seventh days, figure 3). After four days of storage, the MEJA-UVC combination showed significantly higher levels of *trans*-resveratrol (7.23 mg/Kg f.w., figure 3 and figure 4A), piceatannol (1.95 mg/Kg f.w., figure 4C) and total stilbenes (10.03 mg/Kg f.w., figure 4G) than the other treatments. This was not observed for viniferins level (0.84 mg/Kg f.w., figure 4E), which were significantly lower than in the other treatments on the same day (fourth day). However, UVC treatment

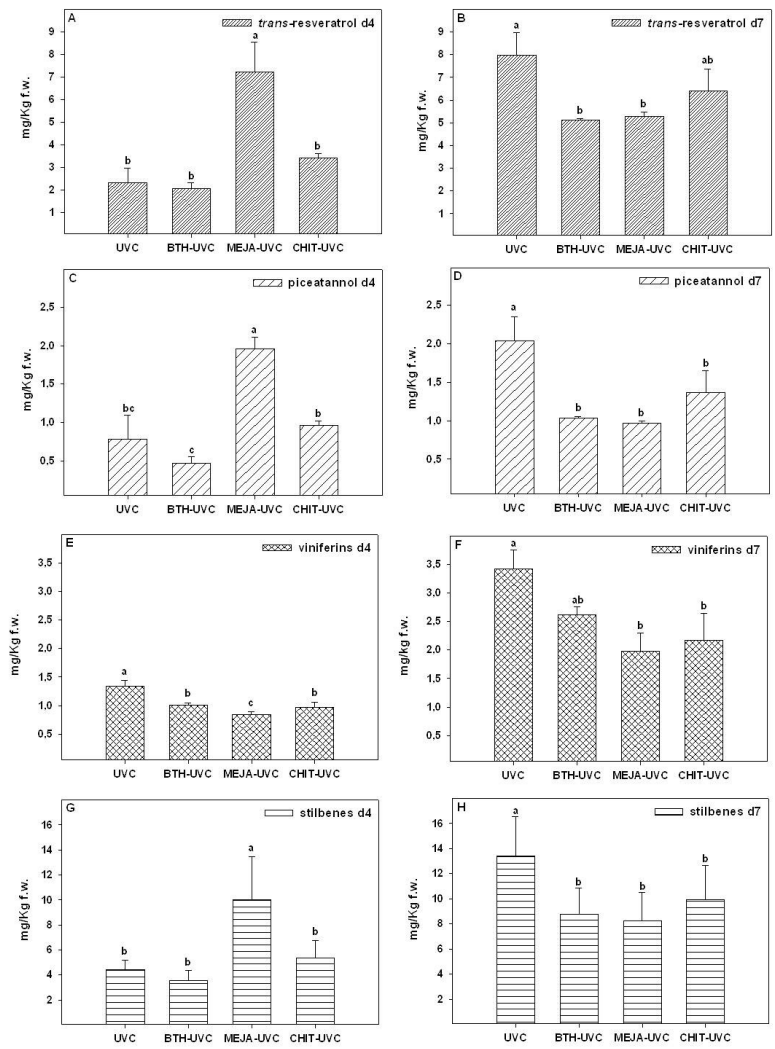

Figure 4 - *trans*-Resveratrol (A, B), piceatannol (C, D), viniferins (E, F) and total stilbenes (G, H) on the fourth (d4) and seventh (d7) days after UVC postharvest treatment. Different letters show statistically different values at p≤0.05. *trans*-Resveratrol, piceatannol, viniferins and total stilbenes on the fourth (d4) and seventh (d7) days after UVC postharvest treatment. Different letter showed statistically different values.

alone (without combination) reached the highest *trans*-resveratrol (7.97 mg/Kg f.w., figure 3 and figure 4B), piceatannol (2.04 mg/Kg f.w., figure 4D), viniferins (3.42 mg/Kg f.w., figure 4F) and total stilbenes (13.43 mg/Kg f.w., figure 4H) concentrations after seven days of storage, in agreement with previous studies developed in our lab (Guerrero *et al.*, 2010a). We noted that the highest *trans*-resveratrol contents were achieved in grapes treated by UVC and by MEJA-UVC. However, the Dm of *trans*-resveratrol and piceatannol was different : it was reduced by three days by the MEJA-UVC combination treatment with respect to the UVC treatment.

A decrease in stilbene concentration was noted in MEJA-UVC at Dm = 7 in comparison with UVC. This reduction was not surprising since it has been described that, after eliciting treatments, stilbenes reach a maximum concentration and then decrease (Guerrero *et al.*, 2010a). Therefore, a same behaviour (decrease) is expected for UVC after the seventh day.

In contrast, BTH-UVC and CHIT-UVC treatments did not achieve a comparable increase in *trans*-resveratrol with UVC during the storage period (seven days) (figures 3 and 4). As for the other stilbenes, lower piceatannol content was observed in both BTH-UVC and CHIT-UVC treatments (figure 4D). A similar viniferin concentration was observed in BTH-UVC but differences were obtained after seven days of storage in CHIT-UVC with respect to UVC (figure 4F). The maximum stilbene concentration with BTH-UVC and CHIT-UVC has only been found after seven days of storage. In contrast with the initial hypothesis, these preharvest treatments did not induce *trans*-resveratrol content with a shorter Dm (probably more than seven days) compared to UVC treatment : they seem to attenuate stilbene induction.

CONCLUSIONS

Two conclusions can be drawn from the results discussed above. Firstly, MEJA and CHIT preharvest treatments are not efficient in increasing *trans*-resveratrol content in grape, at the assayed doses and method. On the other hand, BTH increased *trans*-resveratrol content in grape but this seems to be linked to a ripening delay. Secondly, the combination of the preharvest MEJA treatment and the postharvest UVC treatment (MEJA-UVC) seems to be an interesting application for stilbene-functional grape development. Despite the fact that the grape stilbene concentration reached with the MEJA-UVC combination not exceed that reached by UVC alone, the storage period required after treatment was reduced by three days. This is an important finding because it demonstrates that the combination of MEJA with UVC accelerated stilbene biosynthesis, which is linked to preservation of grape quality. Therefore, the MEJA-UVC treatment is suggested as an interesting application for stilbene-enriched grape production. Functional grapes could have an application as raw material for added-value wines, considering that with the same ingestion of ethanol, the intake of bioactive stilbenes would be significantly increased. Further studies are required to check the influence of both grape variety and year on stilbene biosynthesis.

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